Genetic characterization of striped sea bream (*Lithognathus mormyrus*) populations on both sides of a boundary area between eastern and western Mediterranean basins

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ABSTRACT. - The allozymic analysis was used to investigate intra-species genetic variability and structure in striped sea bream, *Lithognathus mormyrus* (Linnaeus, 1758), captured from marine and lagoon localities belonging to the East of Tunisian coasts. Six polymorphic loci were scored among the thirteen loci identified (Est-1, Est-2, Got-2, Mdh-3, Pgm and Sod). The mean number of alleles per locus, the proportion of polymorphic locus and the mean heterozygosity showed the existence of low variability, which was equally distributed among studied samples. An applied test on the four samples showed significant departure from the Hardy-Weinberg equilibrium (f = 0.243; $p < 0.001$). The interpopulation genetic variability revealed a high degree of genetic homogeneity (*Fst* = 0.017) and the absence of a North/South differentiation.

RÉSUMÉ. - Caractérisation génétique des populations de *Lithognathus mormyrus* de part et d'autre d'une zone charnière entre les bassins oriental et occidental de la Méditerranée.

L'analyse allozymique est utilisée afin d'estimer la variabilité génétique intraspécifique des échantillons marins et lagunaires de *Lithognathus mormyrus* provenant des côtes est de la Tunisie. Dans cette étude, nous avons pu mettre en évidence treize loci dont six sont polymorphes (Est-1, Est-2, Got-2, Mdh-3, Pgm et Sod). Le nombre moyen d'allèles par locus, le taux de polymorphisme et l'hétérozygotie moyenne ont montré l'existence d'une variabilité génétique faible répartie de manière équivalente entre les quatre échantillons étudiés. Un test appliqué sur ces échantillons a montré une déviation de l'équilibre de Hardy-Weinberg (f = 0,243 ; $p < 0,001$). L'analyse de la variabilité génétique interpopulationnelle (*Fst* = 0,017) a révélé l'absence de différenciation Nord/Sud.

Key words. - Sparidae - *Lithognathus mormyrus* - MED - Siculo-Tunisian strait - Allozymes - Genetic variability.

The successful commercial rearing of some species belonging to family Sparidae as *Sparus aurata*, *Diplodus puntazzo* and *D. sargus* (Dujakovic and Glamuzina, 1990), has promoted domestication attempts in other species such as *Dentex dentex* (Glamuzina *et al.*, 1989) and *Lithognathus mormyrus* (Kentouri, 1985). The latter species is a commercially important demersal fish. One of the main features of this fish is its wide geographic distribution. *L. mormyrus* is frequent along Mediterranean coasts, the Atlantic Ocean from Gascogne Gulf to the Cape of Good Hope. It is also found in western Indian Ocean, in Red Sea and in the Black Sea (Bauchot and Hureau, 1990).

Hence, many studies have been carried out about its biology, growth, development, and morphometry (Palma and Andrade, 2002; Türkmen and Akyurt, 2003; Kallianiotis *et al.*, 2005). However, the genetic diversity of *Lithognathus mormyrus* populations is not well known. The genetic studies using allozymic data that have been made about *L. mormyrus* populations in the northern Mediterranean have shown a low genetic differentiation (Arculeo *et al.*, 2003). Whereas, comparing samples from northern Mediterranean coasts with Atlantic samples (Portugal coasts) using separately allozymic and two mitochondrial makers (D-loop and Cytochrome b), have revealed a strong differentiation and a limited gene flow between Atlantic and Mediterranean populations (Bargelloni *et al.*, 2003). Gibraltar strait seems to be then a genetic transition area between two ecologically different waters (hydrology, temperature and salinity).

In this paper, the results of a preliminary study about intrapopulational and interpopulational genetic variability of four *Lithognathus mormyrus* samples, collected from Tunisian coasts are reported. The Tunisian coasts are considered to be a boundary area between eastern and western Mediterranean basins, separated by the Siculo-Tunisian strait. It is the first genetic survey carried out about the south-eastern Mediterranean striped sea bream populations. This study tries to elucidate the degree of differentiation between northeast (western Mediterranean basin) and the southeast (eastern Mediterranean basin) Tunisian populations.

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MATERIAL AND METHODS

Sampling

A total of 150 specimens of *Lithognathus mormyrus* (Linnaeus, 1758), were collected from the north and the south of Tunisian coasts (Tab. II). Samples of populations were gathered from four localities: three lagoons (Bizerta, Ghar El Melh and El Biban lagoons) and one marine site (Tunis gulf) (Fig. 1). Samples of liver and white skeletal muscle were collected from all individuals. Frozen samples were then homogenized with an Ultra-Turrax in an equal volume of extraction solution (ph 6.8). The homogenates were centrifuged at 15000 rpm for 30 minutes at 4°C. Fractions containing soluble proteins were stored at -20°C until electrophoresis.

Electrophoresis

Electrophoresis was performed on potato starch gel using two buffers: Tris-citrate 8.0 and Tris-citrate 6.7 according to Pasteur *et al.* (1987). Thirteen isoenzymatic systems (ADH, EST, GLC, GOT, IDH, LDH, MDH, ME, PGD, PGM, PGI, SDH and SOD) were visualised using enzyme-specific stains according to Pasteur *et al.* (1987) techniques. Alleles were designated by their electrophoretic mobilities relative to the cathode mobility of the most common allele, which was designated as 100.

Statistical analysis

Allele frequencies and genetic variability parameters (mean number of alleles per locus for each population and the totality of populations, Am), polymorphic rate at the threshold of 95% (P), observed (Ho) and unbiased expected (He) mean heterozygosity (Nei and Chesser, 1983) were calculated using the Genetix 4.05 software (Belkhir *et al.*, 1996-2004). To reduce the effect of sample size, unbiased estimator of genetic diversity (He) has been used.

The variation in allele frequencies was quantified using Wright's *F*-statistics estimation (1951) calculated according to the Weir and Cockerham variance based method for estimation of the fixation indices (1984). *Fis* estimates departure from Hardy-Weinberg equilibrium (HWE) for each population; *Fst* measures the degree of genetic differentiation among the different samples. Genetic distances (*D*: Nei, 1978) between samples were also calculated. The Wright indices (*Fst, Fis*) and their statistical significance of departure from zero (tested using 1000 permutations) were assessed by Genetix 4.05 software (Belkhir *et al.*, 1996-2004). Bonferroni adjustment of the *P-values* was used to correct multiple tests (Rice, 1989).

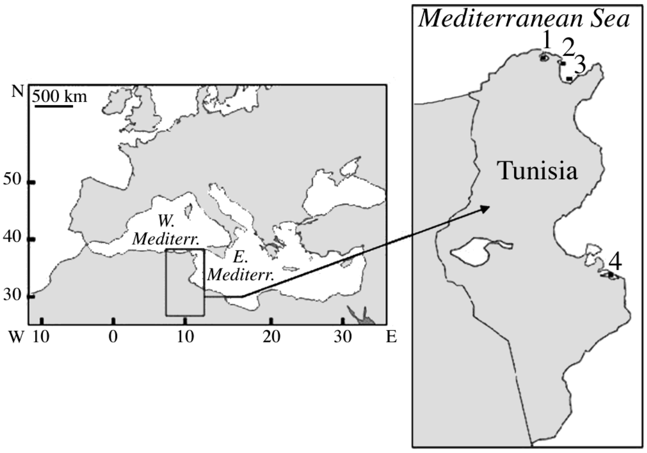

Figure 1. - Sampling localities of *Lithognathus mormyrus*: (1) Bizerta lagoon, (2) Ghar el Melh lagoon, (3) Tunis gulf and (4) El Biban lagoon. [*Sites d'échantillonnage.]*

RESULTS

Among the thirteen tested enzymatic systems (ADH, EST, GLC, GOT, IDH, LDH, MDH, ME, PGD, PGM, PGI, SDH and SOD), only eight gave clear zymograms: EST, GOT, IDH, LDH, MDH, PGM, PGI and SOD (Tab. I). These isoenzymes have shown thirteen loci among them seven were monomorphic (Got-1, Idh-1, Ldh, Mdh-1, Mdh-2, Pgi-1 and Pgi-2) and six were polymorphic (Got-2, Est-1, Est-2, Mdh-3, Pgm and Sod). Each polymorphic locus was represented by two or three alleles (Tab. II).

Allele frequencies of the polymorphic loci (Am) are listed in table II. The average number of alleles per locus was 1.64, it varies from 1.54 to 1.69. At a 95% level, the percentage of polymorphic loci within each sample ranged from 23.08% to 30.77%. The lowest value was observed in the El Biban lagoon and Tunis gulf samples.

Observed (Ho) and expected (He) heterozygosity for

Table I. - Enzyme nomenclature, number of enzyme commission, tissue analysed (M = muscle; L = liver), buffers used and number of loci detected. TC 8: Tris-citrate pH 8; TC 6.7: Tris-citrate pH 6.3 (electrode), pH 6.7 (gel) according to Pasteur *et al.* (1987). *[Nomenclature des enzymes, numéro de commission des enzymes, nombre de loci détectés, tissus étudiés (M = muscle; F = foie) et solutions tampons utilisées : TC 8 : Tris-citrate pH 8 ; TC 6.7 : Tris-citrate pH 6.3 (électrode), pH 6.7 (gel) selon Pasteur* et al. *(1987).]*

Enzymes	E.C.N°	Tissues	Buffers	Loci
Aspartate aminotransférase (GOT)	2.6.1.1	L	TC 6.7	2
Esterase (EST)	3.1.1.2	L	TC 8	2
Isocitrate deshydrogenase (IDH)	1.1.1.42	M & L	TC 8	1
Lactate deshydrogenase (LDH)	1.1.1.27	M	TC 8	1
Malate deshydrogenase (MDH)	1.1.1.37	M & L	TC 6.7	3
Phosphoglucomutase (PGM)	2.7.5.1	M & L	TC8 & TC 6.7	1
Phosphogluco-isomerase (PGI)	5.3.1.9	M & L	TC 8	2
Superoxyde dismutase (SOD)	1.15.1.1	Liver	TC 8	1

Table II. - Allele frequencies; parameters of genetic diversity: mean number of alleles per locus (Am), proportion of polymorphic loci under a 95% criterium (P95%), observed (Ho) and unbiased expected (He) heterozygosity for each locus and population of *Lithognathus mormyrus* with 95% confidence intervals (Nei et Chesser, 1983). Weir and Cockerham (1984) *Fis* values for each locus and each sample of *Lithognathus mormyrus*. NS: Non-significant value after sequential Bonferroni correction; *p < 0.05 ** p < 0.01; *** p < 0.001. *[Fréquences alléliques; estimateurs de la diversité génétique : nombre moyen d'allèles par locus (Am), taux de polymorphisme au seuil de 95% (P95%), hétérozygotie observée (Ho) et hétérozygotie attendue non biaisée (He) pour chaque locus et chaque échantillon avec intervalles de confiance au seuil de 95% (Nei et Chesser, 1983). Valeurs de* Fis *selon Weir et Cockerham (1984) pour chaque locus et pour chaque échantillon ; NS : valeurs non significatives après la correction séquentielle de Bonferroni ; *p < 0,05 ** p < 0,01; *** p < 0,001.]*

		Northern populations			Southern population	
Locus	Samples	Tunis Gulf	Ghar El Melh lagoon	Bizerta lagoon	El Biban lagoon	All samples
	Nb	41	32	45	32	150
Est-1	85	0.012	0.031	0.033	0.031	
	100	0.878	0.937	0.888	0.921	
	110	0.110	0.031	0.077	0.046	
	He	0.219	0.121	0.205	0.149	
	Ho	0.146	0.062	0.177	0.031	
	Fis	0.336	0.488	0.134	0.793 *	0.371***
Est-2	93	0	0.187	0.100	0.062	
	100	0.817	0.796	0.744	0.921	
	105	0.182	0.015	0.155	0.015	
	He	0.302	0.334	0.416	0.148	
	Ho	0.219	0.093	0.333	0.093	
	Fis	0.277	0.723**	0.201	0.372	0.359***
Got-2	71	0.244	0.265	0.333	0.109	
	80	0.036	0.109	0.055	0.171	
	100	0.719	0.625	0.611	0.718	
	He	0.426	0.535	0.518	0.448	
	Ho	0.268	0.375	0.555	0.312	
	Fis	0.374	0.303	-0.073	0.307	0.200
Mdh-3	74	0.024	0.031	0.044	0	
	100	0.975	0.968	0.955	1	
	He	0.048	0.061	0.085	0	
	Ho	0.048	0.062	0.088	0	
	Fis	-0.013	-0.016	-0.035		-0.024
Pgm	100	0.963	0.937	0.977	0.968	
	136	0.036	0.062	0.022	0.031	
	He	0.071	0.119	0.043	0.061	
	Ho	0.073	0.062	0.044	0.062	
	Fis	-0.026	0.479	-0.011	-0.016	0.159
Sod	68	0.024	0.031	0.066	0	
	100	0.975	0.968	0.933	1	
	He	0.048	0.061	0.125	0	
	Ho	0.048	0.062	0.133	0	
	Fis	-0.013	-0.016	-0.060		-0.040
Total loci	Am	1.62	1.69	1.69	1.54	1.64
	P (95%)	23.08	30.77	30.77	23.08	26,92
	He	0.086±0.073	0.095±0.085	0.107±0.090	0.062±0.067	0.087±0.016
	Ho	0.062±0.048	0.055±0.053	0.102±0.089	0.038±0.046	0.064±0.023
	Fis	0.282*	0.421***	0.045 NS	0.385***	0.243***

each locus and each sample are shown in table II. The global sample expected heterozygosity is equal to 0.087 ± 0.016. The lowest level was noted in the southern population (El Biban lagoon; He = 0.062 ± 0.067).

An applied test on the four samples showed significant departure from Hardy-Weinberg equilibrium (*Fis* = 0.243;

Table III. - Pairwise *Fst* values between pairs of samples estimated according to Weir and Cockerham (1984) in samples of *Lithognathus mormyrus* and genetic distances (*D*) between pairs of samples. NS: Non-significant value after sequential Bonferroni correction; *p < 0.05; **p < 0.01; *** p < 0.001 correction. *[Matrice des valeurs de* Fst *par paire d'échantillons de* Lithognathus mormyrus *calculées selon Weir et Cockerham (1984) et distance génétique (*D*) par paire d'échantillons. NS : Valeur non significative après la correction séquentielle de Bonferroni ; *p < 0,05 ; **p < 0,01 ; ***p < 0,001.]*

Fst *D*	Tunis Gulf	Bizerta lagoon	Ghar El Melh lagoon	El Biban lagoon
Tunis Gulf		0.003 NS	0.019 NS	0.026 NS
Bizerta lagoon	0.001 NS		0.002 NS	0.044**
Ghar El Melh lagoon	0.002 NS	0.001 NS		0.014 NS
El Biban lagoon	0.002 NS	0.005*	0.002 NS	

$p < 0.001$). Among the whole sample, only the Bizerta lagoon appears to be in equilibrium for all loci. However, the other samples reveal heterozygote deficiency. The *Fis* values for each locus show heterozygote deficit for loci Est-1 and Est-2.

Among the four samples, *Fst* value (0.017) calculated according to Weir and Cockerham (1984) is non-significant. The computation of Nei's distance (Nei, 1978) between samples gave low values ranging from 0.001 to 0.005 (Tab. III).

DISCUSSION

The purpose of this preliminary study was to obtain a general view of *Lithognathus mormyrus* genetic variability along the Tunisian coasts and especially on both sides of a boundary area between eastern and western Mediterranean basins. The position of Tunisian coasts is interesting, since it is an area where two water bodies with different hydrology, physical and chemical characteristic meet (Ovchinnikov, 1966). This zone seems to be a transition area for some species like *Engraulis encrasicolus* (Bembo *et al.*, 1996), *Dicentrarchus labrax* (Bahri-Sfar *et al.*, 2000) and *Sparus aurata* (Ben Slimene *et al.*, 2004). In this context, analysing populations of marine organisms like *Lithognathus mormyrus* is of particular interest, as it allows us to investigate the consequences of divergent biotic and abiotic conditions on population's differentiation.

The analysis of allozymic variability of Tunisian coast *Lithognathus mormyrus* populations showed a low level of genetic variability. The average number of alleles per locus (Am = 1.64) seems to be comparable with those previously scored by other authors for the same species in Spanish coasts (Am = 1.16; Alarcon *et al.*, 1999), in Greek and Italian coasts (Am = 1.54; Arculeo *et al.*, 2003) and in Portugal, Spain, Italian and Greek coasts (Am = 1.13; Bargelloni *et al.*, 2003). The mean number of alleles per polymorphic locus is dependant on the sampling size and on the geographic distribution (Shriver *et al.*, 1993). For these reasons, it is not the best measure of genetic diversity.

The average expected heterozygosity (He = 0.087 ± 0.016) found in the present study seems to be slightly higher than the average values obtained by Arculeo *et al.* (2003) with Italian and Greek populations of *Lithognathus mormyrus* (He = 0.072 ± 0.003) and it is largely higher than those obtained by Bargelloni *et al.* (2003) on Spanish, Portuguese, Italian and Greek populations of the same species ($0.007 \leq He \leq 0.018$). To clarify the latter statement, a comparison must be made on the basis of the same loci for the two inshore populations. Because the data of Bargelloni *et al.* (2003) are non available, this comparison is only possible with those of Arculeo *et al.* (2003). Based on common loci, genetic diversity seems to be equally distributed in northeast (He = 0.095 ± 0.017; Arculeo *et al.*, 2003) and southeast Mediterranean inshore (He = 0.093 ± 0.003; present study).

Significant departure from Hardy-Weinberg equilibrium was observed, corresponding to heterozygote deficiency in Est-1 and Est-2 loci. Many hypotheses can be advanced to explain such a heterozygotes deficit: selective forces against heterozygote genotypes, crossing system, presence of null alleles and populations substructure (Wahlund effect) (Jin and Chakraborty, 1995; Rousset and Raymond, 1995). The latter can be accentuated with gene flow restricted by presence of geographic, ecological or biological barriers. In this work, we are unable to explain the cause of this deficiency but the hypothesis of Wahlund effect can be rejected because of the low value of genetic distances and the differentiation estimator. The differentiation estimator (*Fst*) of Weir and Cockerham (1984) as well as the genetic distances (*D*) among and between the Tunisian samples show non-significant values (*Fst* = 0.017; $0.001 \leq D \leq 0.005$). These results indicate a low differentiation and suggest a high gene flow between northern (western Mediterranean basin) and southern (eastern Mediterranean basin) samples. They show the existence of a single *Lithognathus mormyrus* population along the Tunisian coasts.

The Siculo-Tunisian strait does not seem to be a geographic barrier to the dispersion of this species. This homogeneity was already pointed out by Arculeo *et al.* (2003) and Bargelloni *et al.* (2003) using allozymes and other molecular markers (mitochondrial DNA D-loop region and the cytochrome b gene).

However, the pairwise *Fst* estimated for the Bizerta and El Biban lagoon samples revealed the existence of genetic differentiation. This heterogeneity is due to the absence of some alleles (Mdh3-74 and Sod 68) in El Biban lagoon sample and to the existence of differences in the allele frequencies values for Est-2 and Got-2.

Indeed our study is preliminary, because we used only four samples of population and the isoenzyme loci generally exhibit a low level of variability. Moreover, Palma and Andrade (2002) stated the existence of significant degree of morphological dissimilarity between Spanish, Italian and Greek populations. So the use of additional samples along the southern inshore areas and the use of more variable markers than allozymes, such as microsatellites, are primordial to increase the significance of our results.

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